JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2115–2118 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2115–2118.2002

Laboratory Diagnosis of Human Neurocysticercosis: Double-Blind Comparison of Enzyme-Linked Immunosorbent Assay and Electroimmunotransfer Blot Assay

J. V. Proaño-Narvaez,[1] Antonio Meza-Lucas,[2] Olga Mata-Ruiz,[2] Roberto Carlos García-Jerónimo,[2] and Dolores Correa[2]*

Hospital de Especialidades del Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City,[1] and Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaría de Salud de México, Mexico City,[2] Mexico

Received 7 December 2001/Returned for modification 22 January 2002/Accepted 13 March 2002

Neurocysticercosis is a common disease in underdeveloped countries. Its diagnosis is based on clinical, imaging (tomography or magnetic resonance), epidemiological, and laboratory data. Several methods based on the detection of antibodies against cysticerci in cerebrospinal fluid or serum have been tested. Among them, an enzyme-linked immunosorbent assay (ELISA) based on the use of a crude parasite antigen has been used by the laboratory network of cysticercosis in Mexico, which has given support to clinicians for up to 7 years. A *Taenia solium*-specific glycoprotein-based electroimmunotransfer blot (EITB) assay was reported to be highly sensitive and specific for this purpose. In order to compare both techniques, we studied 100 neurocysticercosis patients and 70 neurological noncysticercosis controls and searched for specific antibodies in paired samples of serum and cerebrospinal fluid using both techniques. We found that the EITB assay is more sensitive than the ELISA, especially when serum is being tested. Both techniques are more sensitive in cases with multiple living cysts than in cases with single cysts or calcified lesions. No global differences among cases with parasites located in different parts of the central nervous system were found. In the patients with cysts within the parenchyma, the sensitivity of the EITB assay was higher with serum than with cerebrospinal fluid. The immunodominant bands were found to be the same as those previously reported, i.e., GP-39 to -42, GP-24, and GP-13. Based on these results, we suggest the use of the EITB assay in routine diagnosis of cysticercosis for clinical cases.

Taeniasis and cysticercosis caused by *Taenia solium*, the pork tapeworm, are widespread infections in Latin America, Africa, and Asia (6). The disease in humans (neurocysticercosis [NC]) is caused by the metacestode, which develops within the central nervous system. It is often disabling and sometimes fatal. Diagnosis of cysticercosis is suggested by clinical, epidemiological, and serological findings (3), but magnetic resonance imaging or computed tomograpy (CT) scans are the most sensitive and specific diagnostic tools (14). To support them, several laboratory methods have been standardized, which are based on the detection of antibodies against cysticerci or parasite antigens in the cerebrospinal fluid (CSF) or the serum (2). Among them, the electroimmunotransfer blot (EITB) assay developed at Centers for Disease Control and Prevention (15) has the highest sensitivity in serum. This test develops up to seven glycoprotein bands which are specific for *T. solium* cysticercosis.

In Mexico, NC was recognized as a problem of public health several years ago (7). The Central Laboratory of the National Network for Diagnosis of Cysticercosis (10) gives support to clinicians of second- and third-level hospitals of the country to diagnose this disease and to epidemiologists for field studies. The method that has been used up to now is an enzyme-linked immunosorbent assay (ELISA) which is based on the use of a crude antigenic extract of the parasite and has a lower sensitivity in serum than it does in CSF (4). Even so, this technique is easy to perform and is convenient for quality assurance procedures in a laboratory network. In order to incorporate the best technology available into routine diagnosis, the EITB assay was adapted and compared with the ELISA, so the performance was simultaneously tested for both assays. Here we report the results of this comparison and some characterization of the response of the patients in relation to number, type, and localization of parasites within the brain.

MATERIALS AND METHODS

One hundred patients with NC and 70 controls with other neurological disorders were prospectively studied in the Neurocysticercosis Clinic of the Medical Research Unit for Neurological Diseases at the Hospital of Specialties of the Medical Center of the Social Security Institute of Mexico. Ninety-four NC cases were diagnosed according to the consensus criteria recently proposed (3): 15 of them by surgical removal of the parasites (absolute criterion) and the rest by magnetic resonance imaging and CT scan images compatible with cystic NC, plus positive response to cestocidal treatment (two major and one minor criteria). The six cases with calcifications were corroborated by CT scans exclusively, since this method has been shown to be the best in terms of sensitivity and specificity (14). The patients were grouped according to the number, type, and localization of the parasites. The age range was 17 to 71 years (median = 38 years) and 16 to 96 years (median = 44 years) while gender distributions were 50 males and 50 females and 36 males and 34 females for cases and controls, respectively.

Paired samples of serum and CSF were obtained from all individuals at admission and before any treatment was given. The samples were coded and analyzed by an independent person at the laboratory, by ELISA according to the method of Espinoza et al. (4), and by EITB assay as reported by Tsang et al. (15).

* Corresponding author. Present address: 8vo. Piso, Torre de Investigación, Instituto Nacional de Pediatría, SSA, Av. Insurgentes Sur 3700-C, Col. Ins. Cuicuilco CP 03500, México D.F., Mexico. Phone: (525) 5606-0002, ext. 455. Fax: (525) 5506-9455. E-mail: mcorreaa@sni.conacyt.mx.

TABLE 1. Diagnostic performance of ELISA and EITB for cysticercosis

Parameter	Value obtained by method with specimen			
	ELISA		EITB	
	Serum	CSF	Serum	CSF
% Sensitivity	41.0	71.0	86.0	86.0
% Specificity	95.7	95.7	92.8	92.8

The first of these methods is based on the use of a crude extract of the metacestode, at 1 μg/ml, with serum samples diluted 1:1,000 and CSF samples diluted 1:10, and samples are developed with commercial alkaline phosphatase-conjugated anti-human immunoglobulin G. The cutoff point is routinely calculated as the mean of the negative controls of each plate, plus 3 times the standard deviation of at least 50 serum samples from healthy people or of CSF samples from noncysticercosis neurological patients. For EITB assay, *T. solium*-specific glycoproteins are isolated by lentil-lectin-affinity chromatography (15), subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions, and transferred to nitrocellulose membranes. Membrane strips are incubated with serum samples diluted 1:50 or CSF samples diluted 1:10. The reaction is completed with peroxidase-conjugated anti-human immunoglobulin G and H_2O_2-diaminobenzidine as substrate-chromogen. The development of one of the seven specific bands on the nitrocellulose membrane is sufficient to consider the sample positive.

Chi-square or Fischer exact tests were used to determine statistical significance of differences found in frequency (5).

RESULTS

The efficacy of ELISA and EITB tests for diagnosis of NC was evaluated (Table 1). EITB assay is more sensitive although less specific than ELISA in both samples. In ELISA CSF was more useful for diagnosing cases, while EITB assay was equally sensitive using either sample. The performance of the tests also depended on the number and type of lesions (Table 2). As expected, both tests had higher sensitivity in cases with multiple lesions compared to those with a single cyst, and much lower sensitivities were observed when the patients harbored calcified (i.e., dead) parasites.

The predictive values were calculated for the present study and for the prevalence reported for two other populations: neurological patients at a reference hospital (8.6%) and autopsies at a general hospital (2.4%) (12). The positive predictive value was higher for EITB assay in serum and lower in CSF, compared to ELISA. However, it remained high in both samples. As expected, this parameter decreases when the prevalence is lower, and the differences between ELISA and EITB assay became more evident (Fig. 1A).

The negative predictive value of ELISA was considerably low in both samples of the patients studied herein. At the lower prevalence this parameter augmented to levels close to 100%, and the differences between samples and techniques disappeared (Fig. 1B).

The EITB assay gives up to seven different specific bands with positive samples. The frequency of recognition of these bands by all patients and by subgroups classified according to type and number, or localization of lesions is depicted in Table 3. The more frequently recognized bands by the antibodies of the patients were GP-39 to -42, GP-24, and GP-21. It is also apparent in the table that the group with multiple cysts reacted to the larger number of bands. In cases with single cysts the sensitivity of the test was higher in serum, especially when looking at the GP-39 to -42 and GP-24 bands. Even though the same immunodominant bands were observed, patients with calcified lesions presented a low level of recognition for both samples, but especially serum. As can be seen in the same table, there is a significantly higher recognition of GP-39 to -42 and GP-24 by serum as opposed to CSF for the cases with parasites localized in the brain parenchyma. These differences were not seen in cases with parasites lodged in the subarachnoid space, the ventricles, or multiple sites.

DISCUSSION

The main objective of the present work was to evaluate the more successful test reported up to date in the literature, i.e., the EITB test, for diagnosis of cysticercosis. There is no previous comparison of this method with others routinely used in a blinded study with a large sample of cases and controls. We found some expected results: EITB assay is more sensitive than ELISA, especially in serum (16). This is very important for diagnosis of clinically suspected cases, and more relevant when patients with mild symptoms, who do not normally warrant a CSF sample, are being diagnosed.

A low sensitivity was observed with either technique in cases with low number of cysts or calcified lesions only. This was also reported by Espinoza et al. (4) when they reported the standardization of the ELISA and by Wilson et al. (16) when they further characterized the EITB assay.

The specificity of the EITB assay was lower than the one of the ELISA. The possibility that there were cases of NC within the control group is low, but we could not discard the possibility that they harbored extracerebral cysticerci or had been in contact with the parasite without acquiring infection; values above 7% have been reported in healthy people of the country (8, 13). Also, at least 19% of the persons who harbor an adult *T. solium* tapeworm have specific antibodies against these glycoproteins (1). The positive predictive values calculated for serum of a low-prevalence population (2.4%) are in agreement with the ones found in an open population of a region of hyperendemicity. They reported that ELISA gave a positive predictive value of around 5% and that the EITB assay gave a positive predictive value of 27% (13). These results support the notion that the utility of antibody detection assays in epidemiological studies is restricted to locating transmission "hot spots" and not specific cases. This is a problem to solve for epidemiological studies, rather than for support of clinical diagnosis, since antibody detection is considered confirmatory in

TABLE 2. Sensitivities of tests in relation to type and location of parasites

Patient group	*n*	% of samples positive by:			
		ELISA		EITB	
		Serum	CSF	Serum	CSF
NC with multiple cysts	64	51.6	78.1	92.2	92.2
NC with single cysts	30	36.7	63.3	83.3	80.0
NC with calcified lesions	6	0.0	33.3	33.3	50.0
Total	100	41.0	71.0	86.0	86.0

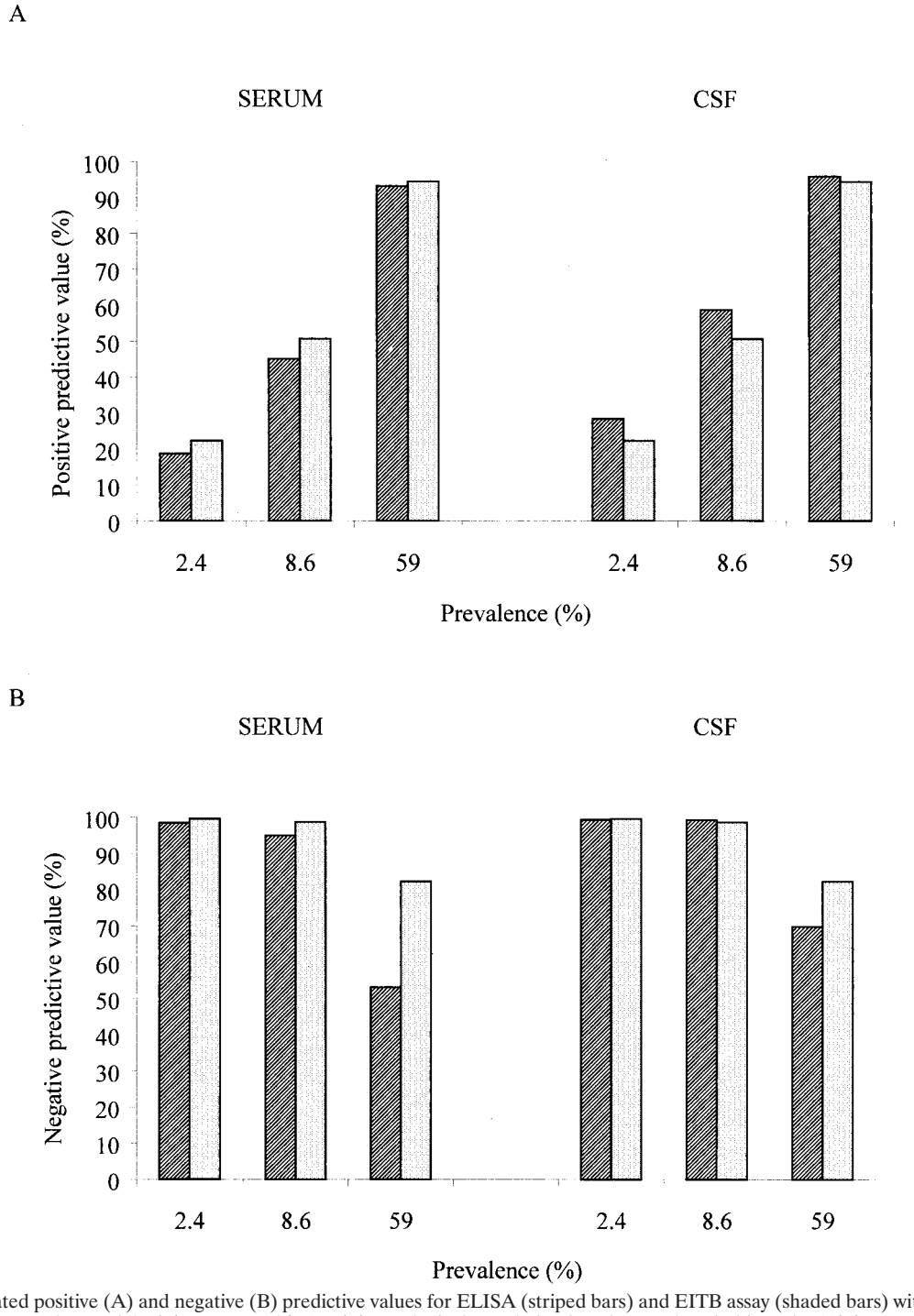

FIG. 1. Calculated positive (A) and negative (B) predictive values for ELISA (striped bars) and EITB assay (shaded bars) with serum and CSF with a cysticercosis prevalence of 59% (present study), 8.6% (neurological population in reference hospitals), and 2.4% (autopsy series in general hospitals).

patients suspected by clinical, imaging, and epidemiological data (3).

The pattern of the glycoproteins found also resulted as expected; GP-39 to -42, GP-24, and GP-13 have been recognized as the immunodominant bands of this test (14). Here we found that the same pattern is obtained regardless of the type, number, or localization of the parasites within the central nervous system.

The EITB assay was more sensitive than the ELISA; thus, we would recommend its use for clinical patients routinely. Also, this technique is the one commonly used in several countries of the world, so it makes it easier to compare data from different regions. Nevertheless, major concerns for the use of this technique are its complexity, time of execution, and cost. While ELISA is based on the use of a crude extract, the EITB assay requires isolation of glycoproteins by affinity chromatog-

TABLE 3. Glycoprotein band recognition in relation to number, type, and localization of cysticerci within the central nervous system

Classification scheme	Sample	Frequency of recognition of the bands (%)						
		GP-50	GP-39–GP-42	GP-24	GP-21	GP-18	GP-14	GP-13
According to type and no. of parasites								
Multiple cysts	Serum	89	92	90	89	55	39	78
	CSF	85	92	90	81	47	47	69
Single cyst	Serum	60	80	83	76	27	33	57
	CSF	61	76	76	60	27	27	43
Calcifications	Serum	33	33	33	33	17	33	33
	CSF	33	50	50	50	33	50	50
According to localization								
Parenchyma	Serum	69	100	100	85	38	38	69
	CSF	62	77	77	46	38	46	46
Subarachnoid space	Serum	74	84	84	84	58	39	71
	CSF	74	84	84	68	45	42	64
Ventricles	Serum	76	76	81	81	38	38	67
	CSF	76	81	81	76	29	29	52
Mixed	Serum	95	97	93	91	49	44	82
	CSF	98	100	97	95	46	53	79
All patients	Serum	77	85	85	82	44	37	69
	CSF	77	85	84	73	40	41	60

raphy, as well as electrophoresis and transfer of proteins. Thus, while ELISA can be accomplished in 20 h and costs around $2.00 per sample, EITB assay requires almost 2 weeks, sophisticated equipment, and highly skilled personnel; thus, it is up to 10 times more expensive. This is especially important for developing countries like Mexico, when dealing with laboratory networks, since quality control is easier to perform in an ELISA format. The efforts made to clone and use the immunodominant bands in ELISA are expected to yield a sensitive but easier and cheaper test (9, 11).

ACKNOWLEDGMENTS

We are thankful to Victor Tsang and his group at the Centers for Disease Control and Prevention, Atlanta, Ga., for training in glycoprotein antigen preparation and for blind comparison of diagnosis of some cases by EITB assay.

REFERENCES

1. **Correa, D., E. Sarti, E., R. Tapia-Romero, R. Rico, I. Alcántara-Anguiano, A. Salgado, L. Valdez, and A. Flisser.** 1999. Antigens and antibodies in sera from human cases of epilepsy or taeniasis from an area of México where *Taenia solium* cysticercosis is endemic. Ann. Trop. Med. Parasitol. **93:**69–74.
2. **Correa, D., J. V. Proaño, J. C. Allan, A. Meza-Lucas, R. Tapia, I. Alcántara, and O. Mata.** 1997. Inmunodiagnóstico de neurocisticercosis mediante la determinación de anticuerpos y antígenos en líquido cefalorraquídeo y suero, p. 279–286. *In* C. Arriagada, J. Nogales-Gaete, and W. Apt (ed.), Neurocisticercosis. Arrynog Ediciones, Santiago, Chile.
3. **Del Brutto, O. H., V. Rajshekhar, A. C. White, Jr., V. C. W. Tsang, T. E. Nash, O. M. Takayanagui, P. M. Schantz, C. A. W. Evans, A. Flisser, D. Correa, D. Botero, J. C. Allan, E. Sarti, A. E. Gonzalez, R. H. Gilman, and H. H. García.** 2001. Proposed diagnostic criteria for neurocysticercosis. Neurology **57:**177–183.
4. **Espinoza, B., G. Ruiz-Palacios, A. Tovar, M. A. Sandoval, A. Plancarte, and A. Flisser.** 1986. Characterization by enzyme linked immunosorbent assay of the humoral immune response in patients with neurocysticercosis and its application in immunodiagnosis. J. Clin. Microbiol. **24:**536–541.
5. **Fleiss, J. L.** 1983. Statistical methods for rates and proportions, 3rd ed., p. 139–162. Wiley, New York, N.Y.
6. **Flisser, A.** 2001. Neurocysticercosis and epilepsy in developing countries. J. Neurol. Neurosurg. Psychiatry **70:**707–708.
7. **Flisser, A.** 1988. Neurocysticercosis in Mexico. Parasitol. Today **4:**131–137.
8. **García, L., M. Torres, D. Correa, A. Flisser, A. Sosa-Lechuga, O. Velasco, A. Meza, A. Plancarte, G. Avila, R. Tapia, L. Aguilar, A. Mandujano, I. Alcántara, Z. Morales, A. Salcedo, M. L. Mañón, and J. L. Valdespino.** 1999. Prevalence and risk factors for cysticercosis and taeniosis in an urban population represented by soldiers and their relatives. Am. J. Trop. Med. Hyg. **61:**386–389.
9. **Greene, R. M., K. Hancock, P. P. Wilkins, and V. C. W. Tsang.** 2000. *Taenia solium*: molecular cloning and serologic evaluation of 14- and 18-kDa related, diagnostic antigens. J. Parasitol. **86:**1001–1007.
10. **Meza Lucas, A., Y. Medina Flores, and D. Correa.** 1994. Formación de una red de diagnóstico de laboratorios de teniasis y cisticercosis. Higiene (Mexico) **II:**199–205.
11. **Plancarte, A., C. Hirota, J. Martínez-Ocaña, G. Mendoza-Hernández, E. Centeno, and A. Flisser.** 1999. Characterization of GP39–42 and GP24 antigens from Taenia solium cysticerci and of their antigenic GP10 subunit. Parasitol. Res. **85:**680–684.
12. **Sarti, E., A. Flisser, D. Correa, J. Sotelo, F. Escobedo, P. Tato, J. Proaño, J. Ambrosio, G. Avila, A. Plancarte, A. S. Aluja, E. Sciutto, J. P. Laclette, W. Willms, and R. Alvarez-Chacón.** 1999. Teniosis y cisticercosis, p. 117–144. *In* J. R. De la Fuente and J. Sepúlveda-Amor (ed.), Diez problemas relevantes en salud pública en México. Fondo de Cultura Económica, México D.F., Mexico.
13. **Schantz, P. M., E. Sarti, A. Plancarte, M. Wilson, J. L. Criales, J. Roberts, and A. Flisser.** 1994. Community-based epidemiological investigations of cysticercosis due to *Taenia solium*: comparison of serological screening tests and clinical findings in two populations in Mexico. Clin. Infect. Dis. **18:**879–885.
14. **Suss, R. A., K. R. Maravilla, and J. Thompson.** 1986. MR imaging of intracranial cysticercosis: comparison with CT and anatomopathologic features. Am. J. Neuroradiol. **7:**235–242.
15. **Tsang, V. C. W., J. A. Brand, and A. E. Boyer.** 1989. An enzyme linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosis of human cysticercosis (*Taenia solium*). J. Infect. Dis. **59:**50–59.
16. **Wilson, M. R., R. T. Bryan, J. A. Fried, D. A. Ware, P. M. Schantz, J. B. Pilcher, and V. C. W. Tsang.** 1991. Clinical evaluation of the cysticercosis enzyme-linked immunoelectro-transfer blot in patients with neurocysticercosis. J. Infect. Dis. **164:**1007–1009.